The structure of triplex DNA in the gas phase

Annalisa Arcella\textsuperscript{a}, Guillem Portella\textsuperscript{a}, Maria Luz Ruiz\textsuperscript{b}, Ramon Eritja\textsuperscript{b}, Marta Vilaseca\textsuperscript{c}, Valérie Gabelica,\textsuperscript{d} and Modesto Orozco\textsuperscript{a,e,*}

\textsuperscript{a} Joint IRB BSC research program in Computational Biology. Institute for Research in Biomedicine. Josep Samitier 1-5. Barcelona 08034. Spain

\textsuperscript{b} Chemistry and Molecular Pharmacology Program. Institute for Research in Biomedicine. IQAC-CSIC, CIBER-BBN. Barcelona 08028. Spain.

\textsuperscript{c} Mass Spectrometry Core Facility. Institute for Research in Biomedicine. Barcelona 08028. Spain.

\textsuperscript{d} Department of Chemistry. University of Liège. Allée de la Chimie, Building B6c, B-4000 Liège, Belgium.


* Correspondence to Prof. Modesto Orozco: modesto.orozco@irbbarcelona.org

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**Table S1.** Summary of simulations performed for 12-mer (charge state -6) and 18-mer triplexes (charge states -7 and -8) in gas phase and aqueous solution for different charge states. See main text for details.

<table>
<thead>
<tr>
<th>Triplex length</th>
<th>Environment</th>
<th># Replicates</th>
<th>Temperature</th>
<th>Length (µs)</th>
<th>Simulation time (µs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-mer</td>
<td>Gas</td>
<td>10</td>
<td>300</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>1</td>
<td>300</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>1</td>
<td>300</td>
<td>1.0 x 5</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>10</td>
<td>372</td>
<td>1.0</td>
<td>10</td>
</tr>
<tr>
<td>Solution</td>
<td></td>
<td>1</td>
<td>300</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>18-mer</td>
<td>Gas</td>
<td>20 (10 replicates for each charge state: -7, -8)</td>
<td>300</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Gas</td>
<td>20 (10 replicates for each charge state: -7, -8)</td>
<td>372</td>
<td>1.0</td>
<td>20</td>
</tr>
<tr>
<td>Solution</td>
<td>1</td>
<td>300</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>Solution</td>
<td>1</td>
<td>373</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>
Table S2: Summary of experimental conditions used on the two Synapt G1 HDMS mass spectrometers.

<table>
<thead>
<tr>
<th></th>
<th>Barcelona</th>
<th>Liège</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Samples</strong></td>
<td>pH = 7, 150 µM triplex, 5% isopropanol – 95% H₂O</td>
<td>pH = 3, 10 µM (12-mer) or 15 µM (18-mer) triplex, 100% H₂O</td>
</tr>
<tr>
<td><strong>-Ionization source</strong></td>
<td>Triversa NanoMate (Advion) chip-based nanoelectrospray source. Spray voltage = 1.65 kV, delivery pressure = 0.9 psi.</td>
<td>Standard electrospray source (Waters, Manchester, UK). Spray voltage = 2.2 kV, flow rate = 4 µL/min</td>
</tr>
<tr>
<td><strong>Source pressure (pirani reading)</strong></td>
<td>5.49 mbar</td>
<td>3.10 mbar</td>
</tr>
<tr>
<td><strong>Cone voltage</strong></td>
<td>40 V</td>
<td>30 V</td>
</tr>
<tr>
<td><strong>Extraction cone</strong></td>
<td>5 V</td>
<td>4 V</td>
</tr>
<tr>
<td><strong>Source temperature</strong></td>
<td>20 °C</td>
<td>25 °C</td>
</tr>
<tr>
<td><strong>Desolvation temperature</strong></td>
<td>not applicable</td>
<td>40 °C</td>
</tr>
<tr>
<td><strong>pₜᵣ in trap and transfer (pirani reading)</strong></td>
<td>4.67 × 10⁻² mbar</td>
<td>2.59 × 10⁻² mbar</td>
</tr>
<tr>
<td><strong>Trap collision energy</strong></td>
<td>10 V</td>
<td>4 V</td>
</tr>
<tr>
<td><strong>Transfer collision energy</strong></td>
<td>10 V</td>
<td>4 V</td>
</tr>
<tr>
<td><strong>IMS cell bias</strong></td>
<td>20 V</td>
<td>10 V, 15 V, 20 V, 25 V, and 30 V</td>
</tr>
<tr>
<td><strong>pₚᵣ in IMS cell (pirani reading)</strong></td>
<td>0.546 ± 0.002 mbar</td>
<td>0.532 ± 0.001 mbar</td>
</tr>
<tr>
<td><strong>Transfer wave velocity</strong></td>
<td>248 m/s</td>
<td>248 m/s</td>
</tr>
</tbody>
</table>
Table S3. Oligonucleotides used as calibrant ions and their published collision cross sections (Ω).

<table>
<thead>
<tr>
<th>Oligonucleotide name</th>
<th>Charge</th>
<th>m/z</th>
<th>Ω in Å²</th>
<th>Reference((^{(a)}))</th>
<th>Used in Liège</th>
<th>Used in Barcelona</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single strand d(TGT)</td>
<td>-1</td>
<td>874</td>
<td>238</td>
<td>JASMS, 2003, 14, 161</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Single strand d(GTT)</td>
<td>-1</td>
<td>874</td>
<td>240</td>
<td>JASMS, 2003, 14, 161</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Single strand dT(_{10})</td>
<td>-3</td>
<td>992</td>
<td>446</td>
<td>JACS, 1997, 119, 9051</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Single strand dT(_{10})</td>
<td>-4</td>
<td>744</td>
<td>537</td>
<td>JACS, 1997, 119, 9051</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Single strand dT(_{10})</td>
<td>-5</td>
<td>595</td>
<td>627</td>
<td>JACS, 1997, 119, 9051</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Single strand dT(_{10})</td>
<td>-6</td>
<td>496</td>
<td>641</td>
<td>JACS, 1997, 119, 9051</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Duplex [d(CG)(_{2})+Na]</td>
<td>-3</td>
<td>789</td>
<td>352</td>
<td>JACS, 2004, 126, 15132</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Duplex [d(CG)(_{3})]</td>
<td>-3</td>
<td>119</td>
<td>430</td>
<td>JACS, 2004, 126, 15132</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Duplex [d(CG)(_{11})]</td>
<td>-11</td>
<td>122</td>
<td>1404</td>
<td>JASMS, 2007, 18, 1188</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Quadruplex [(dTGGGGT)(<em>{4})+NH(</em>{4})(_{3})]</td>
<td>-5</td>
<td>149</td>
<td>775</td>
<td>IJMS, 2006, 253, 206</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Quadruplex dGGG(TTAGGG)(_{3})</td>
<td>-4</td>
<td>166</td>
<td>688</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Quadruplex dGGG(TTAGGG)(_{3})</td>
<td>-5</td>
<td>133</td>
<td>718</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

Table S3 (continued).

<table>
<thead>
<tr>
<th>Oligonucleotide name</th>
<th>Charge</th>
<th>m/z</th>
<th>Ω in Å$^2$</th>
<th>Reference$^{(a)}$</th>
<th>Used in Liège</th>
<th>Used in Barcelona</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadruplex [dGAGGGTGAGGGAGGTTGGG GAAG•(NH$_4$)$_2$]</td>
<td>-4</td>
<td>1776</td>
<td>701</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-5</td>
<td>1419</td>
<td>757</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-5</td>
<td>1412</td>
<td>696</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-6</td>
<td>1182</td>
<td>807</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-6</td>
<td>1176</td>
<td>900</td>
<td>JACS, 2007, 129, 895</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Quadruplex dimer [(dGAGGGTGAGGGAGGTTGGG GGAAG)$_2$•(NH$_4$)$_3$]</td>
<td>-6</td>
<td>2368</td>
<td>1090</td>
<td>(b)</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Quadruplex dimer [(dGAGGGTGAGGGAGGTTGGG GGAAG)$_2$•(NH$_4$)$_3$]</td>
<td>-7</td>
<td>2030</td>
<td>1098</td>
<td>JACS, 2008, 130, 10208</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Quadruplex dimer [(dGAGGGTGAGGGAGGTTGGG GGAAG)$_2$•(NH$_4$)$_3$]</td>
<td>-8</td>
<td>1779</td>
<td>1113</td>
<td>(b)</td>
<td>X</td>
<td></td>
</tr>
</tbody>
</table>

(b) V. Gabelica, E.S. Baker, M.T. Bowers, unpublished results.
Table S4. Key structural descriptors of the 12-mer and 18-mer triplexes in aqueous solution obtained by averaging the last 50 ns of 100 ns trajectories. For groove nomenclature see Shields et al. (Shields, G. C., Laughton, C. A., & Orozco, M. (1997) J. Am. Chem. Soc. 119, 7463). Distances are in Å and angles in degrees. Standard deviations in the average are also displayed.

<table>
<thead>
<tr>
<th></th>
<th>(d(TC(^\text{+})) - d(GA) \cdot d(TC))_{\text{6}}</th>
<th>(d(TC(^\text{+})) - d(GA) \cdot d(TC))_{\text{9}}</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSd</td>
<td>all</td>
<td>central</td>
</tr>
<tr>
<td></td>
<td>2.3 +/- 0.4</td>
<td>1.5 +/- 0.2</td>
</tr>
<tr>
<td></td>
<td>2.23+/-.033</td>
<td>2.05+/-.029</td>
</tr>
<tr>
<td>% H-bonds (all)</td>
<td>WC</td>
<td>Hoogsteen</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>96</td>
<td>89</td>
</tr>
<tr>
<td>% H-bonds (central)</td>
<td>WC</td>
<td>Hoogsteen</td>
</tr>
<tr>
<td></td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Average phase angle</td>
<td>d(TC(^\text{+}))_{\text{6}}</td>
<td>100 +/- 26</td>
</tr>
<tr>
<td></td>
<td>100 +/- 26</td>
<td>110 +/- 44</td>
</tr>
<tr>
<td></td>
<td>d(GA)_{\text{6}}</td>
<td>139 +/- 28</td>
</tr>
<tr>
<td></td>
<td>139 +/- 28</td>
<td>139 +/- 28</td>
</tr>
<tr>
<td></td>
<td>d(TC)_{\text{6}}</td>
<td>108 +/- 26</td>
</tr>
<tr>
<td></td>
<td>108 +/- 26</td>
<td>116 +/- 45</td>
</tr>
<tr>
<td>Groove width</td>
<td>minor</td>
<td>minor-Major</td>
</tr>
<tr>
<td></td>
<td>12.89 +/- 1.31</td>
<td>12.13 +/- 1.71</td>
</tr>
<tr>
<td></td>
<td>12.89 +/- 1.31</td>
<td>12.13 +/- 1.71</td>
</tr>
<tr>
<td></td>
<td>11.61 +/- 0.65</td>
<td>11.68 +/- 0.53</td>
</tr>
<tr>
<td></td>
<td>11.61 +/- 0.65</td>
<td>11.68 +/- 0.53</td>
</tr>
<tr>
<td></td>
<td>20.57 +/- 0.59</td>
<td>20.59 +/- 0.59</td>
</tr>
<tr>
<td></td>
<td>20.57 +/- 0.59</td>
<td>20.59 +/- 0.59</td>
</tr>
<tr>
<td>Twist</td>
<td>GA</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>31.34 +/- 3.38</td>
<td>31.57 +/- 3.6</td>
</tr>
<tr>
<td></td>
<td>31.34 +/- 3.38</td>
<td>31.57 +/- 3.6</td>
</tr>
<tr>
<td></td>
<td>30.53 +/- 4.15</td>
<td>29.63 +/- 3.6</td>
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<td></td>
<td>30.53 +/- 4.15</td>
<td>29.63 +/- 3.6</td>
</tr>
<tr>
<td>Roll</td>
<td>GA</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>1.74 +/- 5.05</td>
<td>1.83 +/- 4.93</td>
</tr>
<tr>
<td></td>
<td>1.74 +/- 5.05</td>
<td>1.83 +/- 4.93</td>
</tr>
<tr>
<td></td>
<td>3.73 +/- 5.32</td>
<td>2.77 +/- 5.37</td>
</tr>
<tr>
<td></td>
<td>3.73 +/- 5.32</td>
<td>2.77 +/- 5.37</td>
</tr>
<tr>
<td>Slide</td>
<td>GA</td>
<td>AG</td>
</tr>
<tr>
<td></td>
<td>-1.17 +/- 0.36</td>
<td>-1.22 +/- 0.41</td>
</tr>
<tr>
<td></td>
<td>-1.17 +/- 0.36</td>
<td>-1.22 +/- 0.41</td>
</tr>
<tr>
<td></td>
<td>-1.14 +/- 0.53</td>
<td>-1.22 +/- 0.54</td>
</tr>
<tr>
<td></td>
<td>-1.14 +/- 0.53</td>
<td>-1.22 +/- 0.54</td>
</tr>
</tbody>
</table>
Table S5. Theoretical (MD) and experimental estimates of CCS for the triplexes, at bias = 20 V using neutral and acidic conditions. The experimental errors were calculated from the 95% prediction interval of the respective calibration curves, meaning that the true value has 95% chance of lying in this interval. The theoretical error bars were determined by propagation of standard errors in the different estimates obtained from individual trajectories, charge states and temperatures.

<table>
<thead>
<tr>
<th>Triplex</th>
<th>Charge</th>
<th>Theoretical CCS (372K)</th>
<th>Experimental CCS (pH = 7)</th>
<th>Experimental CCS (pH = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12-mer</td>
<td>in solution</td>
<td>10.2±0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-5</td>
<td></td>
<td>9.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-6</td>
<td>10.0±0.3</td>
<td>9.7 ± 1.1</td>
<td>9.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>-7</td>
<td>10.2 ± 1.3</td>
<td>10.6 ± 0.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-8</td>
<td>11.8 ± 1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18-mer</td>
<td>in solution</td>
<td>14.3±0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-6</td>
<td></td>
<td>12.9 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-7</td>
<td>13.2±0.1</td>
<td>13.4 ± 1.3</td>
<td>13.3 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>-8</td>
<td>14.3±0.1</td>
<td>14.2 ± 1.5</td>
<td>14.3 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>-9</td>
<td>15.0 ± 1.8</td>
<td>15.4 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>-10</td>
<td></td>
<td>16.6 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>
Table S6. Key simulation results obtained from the analysis of 10 different charge states of the 12-mer and 18-mer triplexes in the gas phase at different temperatures and total charge states. In every case we report the average RMSd (from solution structure), collision cross section, radii of gyration and the maintenance of hydrogen bonds. When appropriate, the solution values are also given.

18-mer charge state -7

<table>
<thead>
<tr>
<th>Replicas</th>
<th>Av. RMSd(nm)</th>
<th>Av. Rg (nm)</th>
<th>Av. Ccs(nm²)</th>
<th>Watson_Crick</th>
<th>Hoogsteen</th>
<th>Tot</th>
<th>Watson_Crick</th>
<th>Hoogsteen</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>0.23 ± 0.03</td>
<td>1.86 ± 0.03</td>
<td>14.3 ± 0.5</td>
<td>96</td>
<td>89</td>
<td>94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.64 ± 0.02</td>
<td>2.05 ± 0.03</td>
<td>13.6 ± 0.5</td>
<td>88</td>
<td>11</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.68 ± 0.01</td>
<td>2.03 ± 0.01</td>
<td>13.9 ± 0.5</td>
<td>15</td>
<td>8</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.67 ± 0.01</td>
<td>2.05 ± 0.01</td>
<td>13.5 ± 0.5</td>
<td>59</td>
<td>27</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.64 ± 0.02</td>
<td>1.80 ± 0.03</td>
<td>12.9 ± 0.4</td>
<td>91</td>
<td>9</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.44 ± 0.01</td>
<td>1.78 ± 0.01</td>
<td>13.0 ± 0.4</td>
<td>88</td>
<td>24</td>
<td>56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.57 ± 0.02</td>
<td>2.87 ± 0.02</td>
<td>13.2 ± 0.5</td>
<td>26</td>
<td>9</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
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18mer charge state=-8

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12-mer charge state -6

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Figure S1: Collision cross section calibration curves: (A) Barcelona; IMS bias = 20 V. (B) Liège; IMS bias = 10, 15, 20, 25 and 30 V. Calibration of the IMS was done with different oligonucleotides in the two instruments. The calibrants lists are given in Table S3. The arrival times were corrected for the traveling time outside the IMS and the traveling time of one wave in the IMS and transfer, yielding \( t_d' \) values that are plotted against the corrected collision cross sections (corrected CCS), following the protocol of Ruotolo et al (Ruotolo, B. T.; Benesch, J. L.; Sandercock, A. M.; Hyung, S. J.; Robinson, C. V. Nat.Protoc. 2008, 3, 1139-1152). A power law was used to fit the data with Sigmaplot 11.0. The 95% prediction interval of the power calibration was used to estimate the error on the experimental CCS. This is therefore the CCS interval in which one has 95% chances of finding the true value using the prediction of the calibration curve.
Figure S2. Time evolution of selected structural descriptors of the 12-mer (TOP) and 18-mer (BOTTOM) triplexes in aqueous solution. Left panels, root mean square deviation curves (RMSds) from canonical triplex structure. Middle and Right panels correspond to numbers the Watson Crick and Hoogsteen hydrogen bonds for the different base pairs, respectively.
Figure S3. Bidimensional RMSd map for the 30 µs trajectory of the 12-mer triplex in the gas phase. Representative structure of cluster obtained at different simulation frames are displayed, noting the weight of each cluster in the total ensemble.
Figure S4. Time variation of several structural descriptors in the 30 µs trajectory of the 12-mer triplex in the gas phase. TOP-LEFT: Radii of Gyration. TOP-RIGHT: average RMSd (with respect to MD-averaged structure) in one microsecond windows. BOTTOM: number of Watson-Crick and Hoogsteen hydrogen bonds.
Figure S5. Examples of contact maps found in gas phase simulations (300K and 372K) for 12-mer and 18-mer triplexes. Charge states displayed were randomly selected. The reference maps obtained in aqueous solution are shown as reference.